

The University of Chicago

EFFECTS OF X-RAYS IN AN INBRED
STRAIN OF GUINEA-PIGS

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

By
HERLUF HALDAN STRANDSKOV

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EFFECTS OF X-RAYS IN AN INBRED STRAIN OF GUINEA-PIGS

H. H. STRANDSKOV

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EIGHT FIGURES

INTRODUCTION

The effects of x-rays and radium on the heredity transmitted by treated animals have attracted a great deal of attention in recent years. The present study concerns effects of x-rays in a highly inbred strain of guinea-pigs. The investigation was carried on under the direction of Prof. Sewall Wright, to whom the writer is indebted for suggestions and criticisms and also for placing at his disposal a genetically homogeneous strain of guinea-pigs, descended entirely from a single mating in the twenty-third generation of brother-sister matings. The history of this strain since 1906, including the records made by it in various respects, have been reported by Professor Wright and associates and form an invaluable background for the interpretation of possible effects.

PLAN OF THE EXPERIMENT

The scrotal region of male guinea-pigs was exposed to varied dosages of x-rays. A number of the treated males were tested weekly for the presence and motility of sperm. A few were sacrificed for histological studies. Those remaining were mated to untreated females. The date of birth and the size of the litters produced by such matings, and the birth weight, the color, the sex, and the thirty-day weight of the offspring were recorded. The young were also examined for abnormalities. Deaths that occurred before the thirtieth day

after birth were recorded. Any animal surviving that period was considered as raised. The immediate offspring of the treated males are designated X-13-0 individuals. They were mated reciprocally to the control inbred strain or in a few cases with each other. The offspring of such matings are

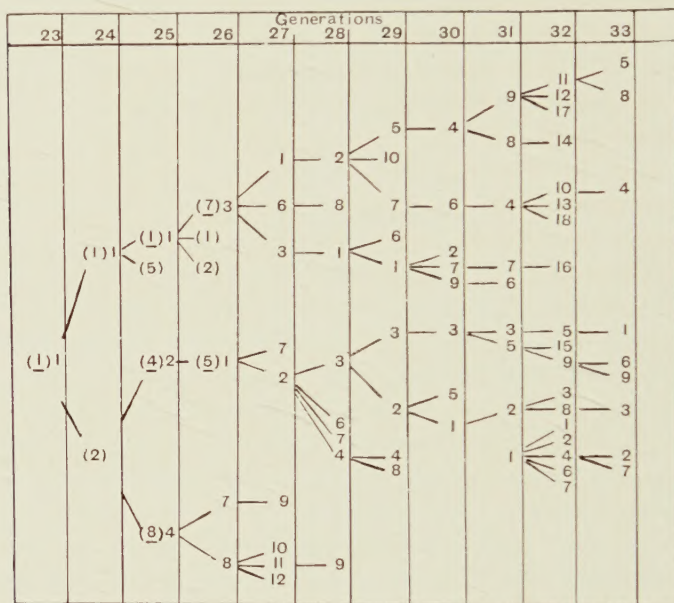


Fig. 1. Pedigree of the line of Fam. 13, as of June 30, 1931, that has descended from a single mating in the 23rd generation of brother-sister matings. The figures represent the mating numbers of a given generation. Those in parenthesis are Washington mating numbers. The matings underlined are those brought to the University of Chicago from Washington by Dr. Sewall Wright.

called X-13-0 F_1 . Brothers and sisters of that generation were mated giving X-13-0 F_2 . Data on mortality and fecundity were recorded for X-13-0 F_1 and X-13-0 F_2 ; also for contemporaneous controls.

History and genetic composition of the animals used

The strain of guinea-pigs used in the present experiment is known as family 13 and is one of a number of inbred strains

that had their origin in experiments of the U. S. Department of Agriculture started in 1906. The treated males and the females to which they were mated were the offspring of parents that had twenty-eight generations of brother-sister matings back of them and all were descended from a single mating in the twenty-third generation. According to Wright ('31), the percentage of heterozygosis under such a system of mating decreases 19.1 per cent per generation. The above strain should accordingly have lost more than 99 per cent of its initial heterozygosis. The homogeneity of the group is evident to any one working with it. It also is borne out by the past performance of the family. (Wright, '22 a and '22 b, also Wright and Eaton, '29). Further evidence is presented by transplantation experiments in which tissues exchanged between individuals of the family behaved practically as autotransplants. (Loeb and Wright, '29).

While the family is vigorous and breeds remarkably well for an inbred strain, it is peculiar in that it normally produces about 5 per cent otocephalic monsters. (Wright and Eaton, '23). Other abnormalities are rare.

Dosage

The male to be treated was firmly fastened down on a lead table with the scrotal area resting over an aperture 6 cm. in diameter. The source of the x-rays was placed directly under the aperture. The x-ray machine was operated at all times at 85 peak kilovolts (alternating potential) and at 5 milliamperes. The target skin distance was 35 cm. The exposure was made without filter. Under the above conditions, the x-ray tube delivered 86.4 Roentgen units per minute, measured in air without back-scattering. The dosage was varied only by varying the exposure time. In the text the dosage is given in minutes.¹

¹I wish to express my appreciation to Dr. P. C. Hodges and Dr. C. S. Capp, of the Division of Roentgenology, Department of Medicine, University of Chicago, for their interest and cooperation in the investigation.

Histological effects

Histological preparations were made for general comparative studies of the effects of x-rays under varied time ex-

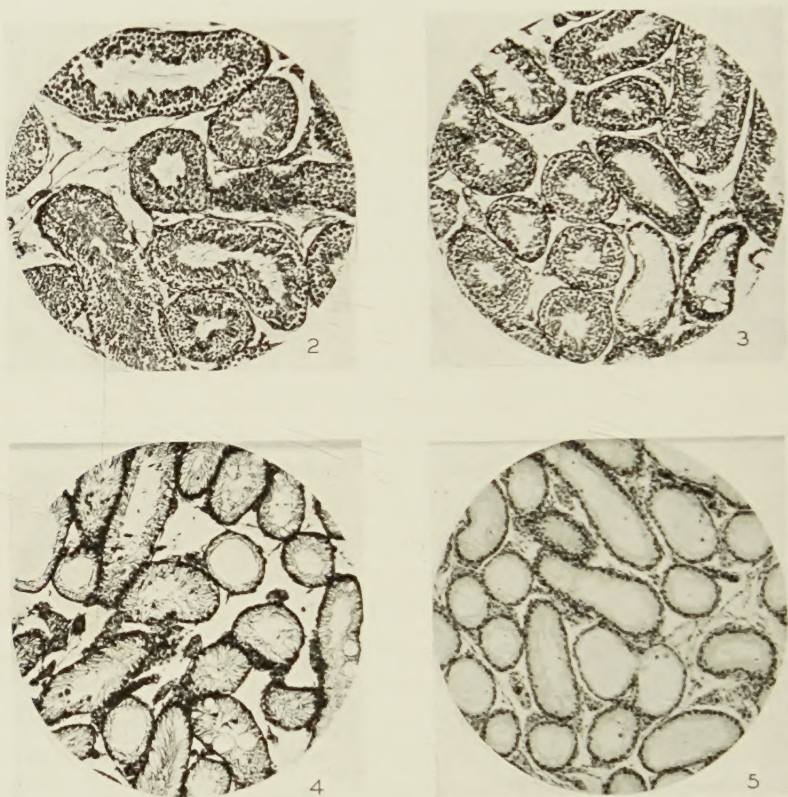


Fig. 2 illustrates normal testis tubules of the guinea-pig.

Fig. 3 Sixteen weeks after ten minutes of raying.

Fig. 4 Seven weeks after twenty minutes of raying.

Fig. 5 Fourteen weeks after thirty minutes of raying.

posures. Figures 2 to 5 are photomicrographs of testis tubules magnified seventy-five times. Figure 2 is a cross-section of normal tubules. Figure 3 is a typical picture of the effect of an intermediate x-ray dosage. The point of special interest in figure 3 is that some of the seminiferous

tubules show marked effects, while others are very little if at all affected. After twenty minutes of raying, all tubules show effects. The clear circular areas shown in the degenerating germinal epithelium, figure 4, are typical for the heavier dosages. The longest exposure made was thirty minutes. After such a treatment, the germinal epithelium is reduced in a few days to a single layer of cells. The regeneration of a new functional epithelium in thirty to forty weeks is evidence that the spermatogonia are not all destroyed.

Sterility

The histological studies gave evidence of a sterility effect. The time of onset and the duration of the sterility period were measured in two ways: first, by examining for motile sperm the seminal fluid emitted following an electric shock (Moore and Gallagher, '30); secondly, by recording the litters produced by females mated to rayed males. Figure 6 summarizes the results of the electric tests. It will be noted that a short period of sterility follows five minutes of raying. After thirty minutes, the sterility period lasts about thirty weeks. In one instance, male X(53) no sterility was noted after fifteen minutes of raying. The only explanation offered is that one or both of the testes may have been shielded by the lead table during the raying.

Figure 7 presents the sterility period as shown by litters produced. An X marks a date sixty-eight days (gestation period) prior to the date of a litter. The picture presented in figure 7 is, of course, not a wholly accurate one, for a considerable period may elapse in guinea-pigs without pregnancy, even though the mated male is not sterile.

The two methods, however, give closely similar results and indicate that approximately one week of sterility is produced under the conditions specified for every minute of raying. After five minutes of raying the sterility period begins only after some twelve to fourteen weeks, but is advanced to the seventh to ninth week with fifteen or more minutes.

X NO.	MINUTES X-RAY	WEEKS																																				
		4	6	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40		
Control																																						
X (61)	2																																					
X (32)	5																																					
X (127)	5																																					
X (129)	5																																					
X (23)	10																																					
X (66)	10																																					
X (125)	10																																					
X (53)	15																																					
X (80)	15																																					
X (131)	15																																					
X (132)	15																																					
X (34)	20																																					
X (64)	20																																					
X (133)	20																																					
X (134)	30																																					

Fig. 6 Sterility periods of irradiated male guinea-pigs as shown by electric tests. — indicates motile sperm, + indicates sperm but no motility, and o indicates no sperm.

MALES	MIN.	WEEK															
		0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
All	2-4			x	x				x	2x					x		
All	5			x					x	x			2x	2x	2x	3x	
All	6-8			x	3x												
All	10	8x	3x			x							3x	x	2x	11x	
X(53)	15	x							x	2x	x						
X(86)	15	x							2x	x						2x	
All																	
other	15		x													11x	
All	20	x														3x	
All	30															22x	

Fig. 7 Sterility of irradiated male guinea-pigs as shown by date of litters. x indicates viable sperm as shown by litters produced, i.e., date of litter minus gestation period (sixty-eight days). The intervals are from date of raying to the end of the second week, to the end of the fourth week, etc.

Matings

Out of a total of eighty-six rayed males, seventy-one were mated. In most cases one treated male was mated to two females, the three animals being placed in a pen $16 \times 24 \times 14$ inches. In a few cases a treated male was mated to four females, the male being shifted at intervals from one pen of two females to another. A summary of these matings with respect to dosage is given in table 1.

As many of the immediate male offspring of rayed males (X-13-0) as space permitted were mated to two females from the untreated control stock. In a few instances males and females of X-13-0 were mated together. Brother-sister mat-

TABLE 1

	MINUTES RAYED										
	2	3	4	5	6	8	10	15	20	30	Total
Males rayed	1	3	2	14	2	3	21	18	9	13	86
Males mated	1	1	1	11	2	2	18	15	8	12	71
Number of X matings	2	2	2	25	4	4	40	39	18	23	159
X matings that produced litters	2	1	2	10	3	1	16	14	3	2	64
Number of litters	2	2	3	15	3	1	32	21	4	2	85
Control 13 litters											140

ings of their young (X-13-0 F_1) were made, giving X-13-0 F_2 . In most of these cases one male was mated to two females.

The control inbred stock was maintained by making brother-sister matings with only one female in a pen.

Thus it will be seen that the control and the experimental matings differed in two respects in addition to the treatment: first in that the experimental pens had two females and one male in them while the control had only one male and one female; secondly, the control were brother-sister matings while the experimental were not, except in the case of the parents of X-13-0 F_2 . Experiences have shown that the last-mentioned difference is of no significance where the animals used are exclusively from a late generation of one highly inbred strain. The difference of one and two females in a

pen is, however, a factor to be considered in connection with some of the data obtained, and an attempt is made to make proper allowance for it. In all other respects, in so far as the writer is aware, the breeding conditions were the same. The animals were kept in a building constructed especially for animal breeding with proper ventilation and with temperature controlled in the winter time.

As shown by table 2 the litters produced per month by the control and the experimental group are quite equally distributed. Thus differences created by seasonal variations should be negligible.

TABLE 2
Distribution of litters

	1929												1930												1931				TOTAL
	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	
Control 13	6	5	5	4	7	11	5	1	4	3	4	6	1	7	5	2	5	5	4	7	2	3	10	3	9	3	9	4	140
Total X-13-0	4	3	2	3	3	4	1	2	3	3	0	3	3	5	7	8	2	10	2	2	1			5	1	2	2	4	85
2 to 8 min.	4	2	2	3	1	2							1	1	4	1		2		1								2	26
10 min.							1	1	3	2		1	2	2	5	1	4	1	1	1			4			1	2	32	
15 to 30 min.		1			2	2	1		1		2	2	2	1	2	1	4	1					1	1	2	1		27	
Control 13							1	4	3	4	6	1	7	5	2	5	5	4	7	2	3	10	3	9	3	9	4	97	
X-13-OF ₁							1		1	2	2	4	2	3	5	4	6	5	3	2	2	8	7	3	9	6	11	86	
Control 13															2	5	5	4	7	2	3	10	3	9	3	9	4	66	
X-13-OF ₂															1	1	2	3	2	4	1	7	5	6	5	9	12	58	

Litter size

Family 13 has been producing an average litter size of about 2.65 since 1915, before which time it was somewhat higher (Wright and Eaton, '29). The rather large annual fluctuations (minimum 2.36 in 1916, maximum 2.89 in 1923), due to environmental conditions, emphasize the necessity of a contemporary control for making comparisons.

For the total eighty-five litters produced in the present experiment by females mated to rayed males the average litter size was $2.04 \pm .097$, which is much lower than any annual

record of the family, while the control averaged $2.69 \pm .079$, in close agreement with its past average.

To determine the significance of the difference of the means the following formula is used—

$$t = \frac{\Delta}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

in which Δ equals the difference of the means, n_1 and n_2 the number of litters and σ_1^2 and σ_2^2 the respective variances

TABLE 3

EXPERIMENT	TOTAL BORN	NUMBER OF LITTERS	S.D.	LITTER SIZE	S.E.	MEAN LITTER ¹
Control 13	376	140	.93	2.69	.079	2.97
Total X-13-0	173	85	.89	2.04	.097	2.41
2 to 8 min.	57	26	1.09	2.20	.214	2.69
10 min.	68	32	.91	2.13	.161	2.69
15 to 30 min.	48	27	.66	1.78	.127	1.80
Before sterility	39	23	1.08	1.70	.225	2.26
After sterility	134	62	.83	2.16	.105	2.47
Control 13	271	97	.90	2.79	.091	3.06
X-13-0 F ₁	238	86	.99	2.77	.107	2.92
Control 13	185	66	.89	2.80	.111	3.05
X-13-0 F ₂	185	59	1.27	3.14	.165	3.45

¹ The mean litter sizes given in this column are based on the young raised and the individual as a unit.

of the two distributions. The probability that random sampling from a homogeneous population would yield a difference as great as that observed is found in tables of the probability integral.

Table 3 gives the number of young born, the number of litters, and the mean litter size for each group; also a column of mean litter sizes based on the young raised to thirty days and on the individual instead of the litter as a unit. These

means are used only in connection with corrections for birth and thirty-day weights.

Table 4 gives the distribution of the litters with respect to size, while table 5 gives the values of *t* for all comparisons made; also the probability of *t*.

Applying the formula mentioned to the mean litter sizes of control 13 (2.69) and X-13-0 (2.04), it is found that the difference has a probability of occurring, as a result of random sampling, of less than one in a million. It remains

TABLE 4

EXPERIMENT	TOTAL LITTERS	DISTRIBUTION OF LITTER SIZE				
		1	2	3	4	5
Control 13	140	17	38	57	28	—
Total X-13-0	85	25	38	17	4	1
2 to 8 min.	26	8	9	6	2	1
10 min.	32	9	12	9	2	
15 to 30 min.	27	8	17	2		
Before sterility	23	12	8	2		1
After sterility	62	13	30	15	4	
Control 13	97	8	27	39	23	
X-13-0 F ₁	86	9	24	34	16	3
Control 13	66	4	22	23	17	
X-13-0 F ₂	59	7	10	14	24	4

to consider how far this difference can be attributed to the raying and how far to the difference in the number of females in a pen. While the present study gives but little data involving such a difference, a paper of Doctor Colin ('31) on lead treatment in guinea-pigs does present some data from which conclusions on this point may be drawn.

By weighting four comparisons in Doctor Colin's data, involving only the difference of one and two females in a pen, according to the formula $\frac{n_1 n_2}{n_1 + n_2}$ in which n_1 and n_2 represent the number of young in the two groups, the following

TABLE 5

GROUPS COMPARED	LITTER SIZE		PER CENT DEAD		PER CENT RAISED		CORRECTED 0 WEIGHT		CORRECTED 30 WEIGHT		SEX RATIO	
	t	P	t	P	t	P	t	P	t	P	t	P
Control 13—X-13-0	5.24	.000	2.46	.02	2.27	.03	2.70	.01	3.50	.001	1.34	.18
Control 13—2 to 8 min.	2.15	.04	.42	.68	.99	.32	2.78	.01	3.80	.000	.06	.95
Control 13—10 min.	3.15	.01	1.90	.06	1.41	.16	1.03	.21	.25	.71	1.59	.11
Control 13—15 to 30 min.	6.11	.000	2.16	.04	2.06	.04	2.05	.04	3.24	.001	.79	.43
Control 13—Before sterility	4.27	.000	.42	.68	.24	.81	.66	.51	3.64	.001	.98	.33
Control 13—After sterility	4.05	.000	2.85	.01	2.55	.02	3.15	.01	2.51	.02	1.12	.26
Before—After sterility	1.89	.06	2.27	.03	1.24	.22	2.46	.02	1.83	.07	.22	.82
Control 13—X-13-0 F ₁	.79	.43	2.56	.02	3.19	.01	1.27	.20	1.10	.27	.34	.74
Control 13—X-13-0 F ₂	1.71	.09	.12	.81	.93	.35	2.24	.03	2.76	.01	1.09	.28

results were obtained: litter size was 0.09 larger for one female in a pen, the percentage born dead was 0.40 larger for one female in a pen; the percentage that died before thirty days was 9.8 smaller for one female in a pen; the percentage raised was accordingly 9.4 larger for one female in a pen while the birth weights and the thirty-day weights were 0.9 grams and 13.4 grams, respectively, larger for one female in a pen than for two. Thus, as might be expected, litter size, prenatal mortality, and birth weight are not appreciably affected by the number of females in a pen, while postnatal mortality is greater and thirty-day weight slightly less with two females in a pen instead of one.

Returning to a consideration of litter size, the absence of an effect from the additional female in a pen makes it seem certain that the observed effect is due to x-rays.

There is another factor in which the x-ray and the control lots differ which affects litter size, viz., the distinctly lower frequency of litters produced by the x-rayed males, but this factor is one which would tend to produce effects in the opposite direction to that observed. According to Wright ('22 b), cross-bred females mated with inbred males produced relatively few litters as compared with similar females mated with cross-bred males (3.82 per year instead of about 4.36). The size of their litters were as a result significantly larger (3.10 and 2.56, respectively), interpreted as due to the greater interval between litters. Other evidence (Wright, '22 a) points in the same direction. Thus, as far as effects of infrequent litters on the females is concerned, those mated with x-rayed males might be expected to produce larger litters than the control, but instead of this we find the marked and significant decrease described above.

If the X-13-0 litters are grouped into those from males rayed less than ten minutes, those from males rayed ten minutes, and those from males rayed more than ten minutes, it is shown in table 3 that the average for the last group differs from that of the other two combined by a little more than two times the standard error of the difference.

With the litters from x-rayed males grouped into those sired prior to the sterility period and those after that period, table 3 shows that the greatest decrease in litter size comes before the sterility period (average 1.70 compared with 2.16). The difference between the two as shown in table 5 is, however, not quite two times its own standard error.

X-13-0 F₁ litters compared with those from the control over the same period of time (table 2) show no appreciable difference in size. The probability that the difference observed might occur between two samples from the same population is one in less than three. Since on a genetic basis no difference is to be expected in this case, while the difference in number of females in a pen is the same as before, agreement in litter size strengthens the conclusion reached above that litter size is not affected by a difference of one and two females in a pen.

In the X-13-0 F₂ the litter size exceeds that of the control, but the excess perhaps only reflects a slight inequality in the distribution of dates of birth of the litters of these groups apparent in table 2.

Percentage born dead

The number 'born dead' means the number of young dead at birth or dead before the daily examination of new litters. Table 6 shows this percentage for the various groups. Comparisons of these percentages were made using the following formula:

$$t = \frac{\Delta}{1.25 \sqrt{\frac{P_1(1-P_1)}{n_1} + \frac{P_2(1-P_2)}{n_2}}}$$

in which Δ equals the difference between the groups compared; P_1 and P_2 , the two percentages,² and n_1 and n_2 , the respective total number of young born.

As pointed out by Wright ('22), the n to be used in this case is neither the litter number nor the total number born,

² In this and other cases it is to be understood that all percentages are reduced to the form of decimal fractions for calculation.

but an intermediate value due to the imperfect correlation between litter mates in survival. n as the total number born is used in these comparisons, but to correct roughly for the above correlation the standard error of the difference is increased 25 per cent.

Table 6 shows that the percentage born dead of X-13-0 is considerably higher than the percentage born dead of the control in spite of the much smaller litter size of the former. The

TABLE 6

EXPERIMENT	TOTAL BORN	PER CENT DEAD	PER CENT DIED	PER CENT RAISED	NUMBER OF OTOS	PER CENT OF OTOS
Control 13	376	14.36	14.10	71.54	23	6.12
Total X-13-0	173	26.01	15.03	58.96	11	6.36
2 to 8 min.	57	17.54	19.30	63.16	3	5.26
10 min.	68	27.94	11.76	60.30	5	7.35
15 to 30 min.	48	33.33	14.59	52.08	3	6.25
Before sterility	39	11.54	19.23	69.23	3	7.69
After sterility	134	29.85	14.18	55.97	8	5.97
Control 13	271	13.65	9.96	76.38	22	8.12
X-13-0 F ₁	238	24.79	15.13	60.08	25	10.50
Control 13	185	15.68	9.73	74.59	15	8.11
X-13-0 F ₂	185	15.13	15.68	69.19	13	7.03

probability that this is due to chance as shown by table 5 is about one in a hundred. Thus it seems probable that the difference reflects an effect of x-rays. The question of number of females in a pen is probably not a factor to be considered, for the data of Colin, as already noted, show a decreased percentage dead with two females in a pen.

The argument that the increase in percentage dead is due to the effects of x-rays is strengthened by the trend shown in tables 5 and 6 when the experimental group is broken up into those of low, intermediate, and high dosage.

The difference in the percentage dead of the group sired prior to the sterility period and those after the sterility period is rather striking. It may, however, be a reflection of the difference in size of litter. As will be recalled, the average litter size of the former was much smaller.

When the young of X-13-0 F₁ and its control are compared for percentage born dead, a difference of 11.14 per cent is found, which is 2.56 times its standard error, a difference which is rather difficult to explain.

X-13-0 F₂ compared with its control shows no difference in percentage dead.

The postnatal mortality does not differ significantly between any of the groups. The percentage raised naturally reflects the results with respect to the prenatal and postnatal mortality.

Birth weight

In dealing with birth weights only those animals which were raised (reached thirty days) are considered. The high mortality of the progeny of the x-rayed males thus has no effect on the birth weights discussed here.

An animal born in a small litter is in general heavier than one born in a large one. It is therefore necessary, in order to compare the birth weights of animals born in litters ranging from one to five in size, to correct the birth weights to a standard litter size. A method for making such a correction was described by Wright and Eaton ('29). It consists in multiplying the mean weights by a correction factor (tabulated in the above paper) designed to adjust the weight to a constant size of litter. In applying the method, the size of litter with the individual as a unit instead of the litter should be used. The mean litter sizes for those animals which reached thirty days are given in table 3 and are the basis of the correction, to a standard litter size of 2.50.

The standard deviations of the corrected birth weights are calculated according to the formula—

$$L^{\sigma} B = \sigma_B \sqrt{1 - r^2} \sqrt{BL}$$

The values of t for differences in corrected birth weights of the different groups are calculated according to the formula—

$$t = \frac{\Delta}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

in which Δ equals the difference between the two corrected birth weights compared; n_1 and n_2 , the respective numbers born in the two groups; and σ_1^2 and σ_2^2 the variances of the corrected birth rates.

Due to the fact that the arithmetic rather than the geometric mean of birth weight is corrected and the mean litter size of X-13-0 F_1 and X-13-0 F_2 and their controls are much larger than the standard 2.50, a slight error creeps in. Therefore, the standard error of the difference of the corrected birth weight in these last two comparisons is increased by the ratio of the average of the two corrected weights to the average of the uncorrected weights. In the case of X-13-0 F_1 and its control, this correction factor is 1.07. In the case of X-13-0 F_2 and its control, it is 1.11.

As shown in table 7, the standard deviation of corrected weights is not calculated for all the groups. 10.99 is used for the corrected birth weights of all the control groups and 10.85 for all the X-13-0 groups. Similarly for the standard deviation of the corrected thirty-day weights.

As shown in table 7, the immediate offspring of rayed males according to the corrected figures are lighter at birth than those from the control, although heavier in the uncorrected average. The difference is 2.70 times its standard error, and has therefore a probability of occurring between two random samples of the same population of less than one in a hundred. It will be recalled from the data of Colin that birth weight is not affected by an additional female in a pen under the conditions of the experiment. Thus the difference observed may reasonably be attributed to x-rays.

It will be seen that in every case the offspring of x-rayed males are inferior to the control, confirming the reality of the difference in the corrected averages.

TABLE 7

EXPERIMENT	10 WEIGHT RAISED	30 WEIGHT RAISED	CORRECTED 0 WEIGHT	S.D. COR. 0 WEIGHT	CORRECTED 30 WEIGHT	S.D. COR. 30 WEIGHT
Control 13	85.22	266.72	90.93	10.99	281.66	42.49
Total X-13-0	88.62	264.66	87.50	10.85	262.01	50.21
2 to 8 min.	83.50	244.17	85.75		249.54	
10 min.	89.15	279.90	89.06		279.62	
15 to 30 min.	95.12	269.16	86.27		248.17	
Before sterility	95.54	251.93	92.38		245.13	
After sterility	86.70	266.53	86.35		265.73	
Control 13	84.17	264.62	90.90		282.35	
X-13-0 F ₁	84.50	264.54	89.57	8.71	277.77	44.72
Control 13	84.86	269.29	91.56		286.79	
X-13-0 F ₂	77.62	242.27	88.41	9.44	270.37	46.30

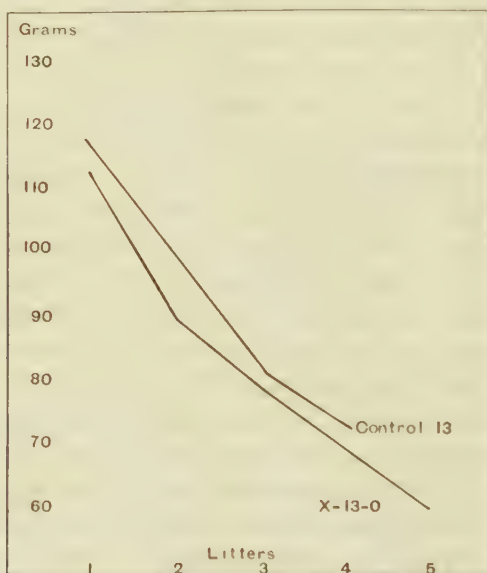


Fig. 8 Average birth weights of young raised in litters of a given size from x-ray and control matings.

There are no significant differences related to amount of dosage.

X-13-0 F₁ showed no significant decrease in birth weight, but the difference in the X-13-0 F₂ and its control is 2.27 times its standard error and thus suggests an effect due to x-rays. It is, of course, in these F₂ young that recessive mutations might be expected to show their effects for the first time.

Thirty-day weights

The thirty-day weights are corrected to a standard litter size of 2.50 in the same manner as are the birth weights, using the correcting tables presented by Wright and Eaton ('29) for thirty-three-day weights. The standard deviations of the corrected thirty-day weights are calculated according to the formula:

$$L \sigma_W = \sigma_{WL} / \sqrt{1 - r_{WL}^2}$$

in which σ_W equals the standard deviation of the uncorrected thirty-day, or weaning, weights, and r_{WL} the correlation of size of litter and weights at thirty days. Values of t for comparisons of the thirty-day weights of different groups are obtained according to the formula given for birth weights.

The average thirty-day weight of a group naturally reflects in part the average weight of the group at birth. Differences between the corrected means of thirty-day weights are, however, in general greater than the differences found in the case of birth weight. This may be accounted for either by an increased manifestation of an x-ray effect on growth or by that of the additional female in a pen, which, as noted, appears to make a difference in this case, although not in birth weight. The difference of 19.6 grams between the control and the progeny of the x-rayed males may be compared with the difference of 13.4 grams in Colin's data in favor of one as compared with two females in a pen. The data confirm the conclusions for the birth weights as far as they go, but cannot be given much significance. Similar considerations apply in the other comparisons.

Sex ratio

The sex distribution in guinea-pigs is in general close to a 1:1 ratio. Family 13 used in the present experiment has not been an exception to this rule, although males during the twenty-eight months that the experiment has been run have been produced in this strain in a slight excess over females. The variation from a 1:1 ratio is, however, not a statistically

TABLE 8

EXPERIMENT	TOTAL BORN	SEX (1)	NUMBER OF MALES	PER CENT MALES	NUMBER OF FEMALES	PER CENT FEMALES
Control 13	376	17	188	52.37	171	47.63
Total X-13-0	173	4	99	58.58	70	41.42
2 to 8 min.	57	3	29	52.70	25	46.30
10 min.	68	1	42	62.69	25	37.31
15 to 30 min.	48	0	28	58.33	20	41.67
Before sterility	39	1	23	60.53	15	39.47
After sterility	134	3	76	58.02	55	41.98
Control 13	271	10	136	52.11	125	47.89
X-13-0 F ₁	238	5	117	50.21	116	49.79
Control 13	185	10	91	52.00	84	48.00
X-13-0 F ₂	185	1	111	60.33	73	39.67

significant one. For a comparison of sex ratios the following formula is used:

$$t = \sqrt{\frac{P_1(1-P_1)}{n_1} + \frac{P_2(1-P_2)}{n_2}}$$

in which Δ equals the difference between the groups compared. P_1 and P_2 are the respective percentages, and n_1 and n_2 the numbers born, respectively, in the two groups.

Table 8 shows that the percentage of males is increased considerably in the immediate progeny of the rayed males, but the deviation is not statistically significant.

A considerable predominance of males is also noticed in X-13-0 F₂, but compared with the control the difference is again not a significant one.

Abnormalities

Very few abnormalities other than the otocephalic monsters which appear normally in family 13 were produced in either the control or the experimental group. Four appeared among the control out of a total of 376 born. Two of these were hydrocephalic and club-footed with the palms of the fore feet strongly flexed and the hind feet stubby. One of them, male no. 20445, had a little toe on each hind foot—a condition common in certain stocks, but never before observed in family 13. The other two listed as abnormals were slightly club-footed, and had palmar flexions of the fore feet.

Two abnormals other than otocephalics appeared in X-13-0. These were hydrocephalic. Four appeared in X-13-0 F₁. One is peculiar in that its penis is duplicated. It survived and has been mated over a year, but has not sired any young. The other abnormalities in this group as well as the one which appeared in X-13-0 F₂ were slight foot defects. There is clearly no indication here of increased tendency to abnormality due to x-rays.

The otocephalic monsters produced in the experimental group were similar to those that appear regularly in the control. In no case does the percentage of otocephalics in the progeny of x-rayed males or in the descendants differ significantly from those of contemporary controls. Both the control and the experimental group were producing a higher percentage during the latter part of 1930 and the early part of 1931 than expected from the previous history of the family. However, no tendency of x-radiation to increase the percentage is apparent.

DISCUSSION

Among the first to examine histologically the effects of x-rays on the testes of mammals were Bergonie and Tribon-

deau ('04). These investigators noted that heavy dosages produced degeneration of all seminiferous tubules, but that more moderate ones affected only a few. They came to the general conclusion that the degree of the effect depends upon the activity of the cells, the more active ones being most severely affected. More detailed studies of the effects of x-rays on seminiferous tubules have recently been made by Gatenby and Wigoder ('29). Their studies were made on the guinea-pig and show that spermatogonia are more resistant than spermatids and spermatocytes. The above results were verified in the present experiments.

Sterility effects following x-ray treatment were first reported by Albers-Schönberg in 1903. Others have noted the same effects. Among the more recent studies are those of Snyder ('25) on the rat. It will be recalled that in the present study sterility effects were produced by all dosages of five or more minutes and that increased dosages regularly brought earlier onset and longer duration of the period of sterility.

Early attempts to produce hereditary effects by radium and x-rays gave negative results. In 1921-1922, Mavor reported a great increase in the frequency of nondisjunction and loss of the X-chromosome in the eggs of *Drosophila* following x-ray treatments. He later described effects on the linkage relations in the same organism.

In 1924, Little and Bagg reported three inheritable abnormalities in the descendants of x-rayed mice, which did not appear in the controls. Muller ('27-'28) reported a definite increase in point mutations and chromosome aberrations in the fruit fly (*Drosophila melanogaster*). Lethals, dominant and recessive, formed the largest class. His findings have been verified by Hanson and Heys ('28), Weinstein ('28), Timofeef-Ressovsky ('29), and others. Whiting ('28) reported mutations by x-rays in the wasp *Habrobracon*. Patterson ('28) was able to produce somatic mutations in *Drosophila*.

Results have also been obtained in plants. Gager and Blakeslee ('27) reported mutations in *Datura* following exposure to radium rays. Goodspeed and Olson ('28) obtained mutations in tobacco by x-ray treatment of sex cells. Stadler ('28) induced numerous mutations in barley and in corn.

In the present study no visible mutations were obtained. The most definite effect observed was a decrease in the size of the litters sired by x-rayed males as compared to the litters of the control. No evidence was obtained as to how the reduction was brought about, but two possible explanations seem to exist—either that fewer eggs were fertilized, due to a deficiency of spermatozoa, or that some of the fertilized eggs developed only through early stages and were then absorbed.

It does not seem likely that a mere reduction in number of spermatozoa could be the determining factor. The electric tests showed numerous motile sperms during the times litters were produced. The more reasonable explanation seems to be that the normal number of eggs was fertilized, but that some of them due to lethal effects were absorbed. Such lethal effects could be due to dominant point mutations or to chromosome aberrations. Such an explanation seems reasonable, in view of the findings of Regaud and Dubreuil ('08) with rabbits. These investigators found in a few cases abnormal embryos being absorbed in the uterus of females that had been mated to rayed males. The studies of Bardeen ('07) and of Hertwig ('11) also make such an interpretation a probable one. Bardeen irradiated toad sperms and found that eggs fertilized by such sperms died after a short developmental period. Hertwig, exposing the sperms of *Rana viridis* and *Rana fusca* to radium, found developing eggs arrested during gastrulation and monstrosities appearing among the embryos. Muller ('28) has obtained evidence of various sorts which indicates a high frequency of dominant as well as of recessive mutations in *Drosophila*.

The trend in the litter size shown in the present experiment with increases in dosage is also in harmony with the theory that the decrease observed is due to lethals produced

by x-rays. Stadler ('29), Oliver ('30), Hanson, Heys, and Stanton ('31) have reported an increase in lethals proportional to the strength of the dosage.

The difference in the decrease of the litters sired before and after the sterility period with a probability of 0.06 may suggest that lethals are most readily produced in mature spermatozoa or spermatids and spermatocytes. However, the decrease of litter size following the sterility period indicates that spermatogonia may also be affected and transmit an effect to the spermatozoa to which they give rise, which permits fertilization but not development. No differential effect may exist, for it is probable that the effect produced in spermatogonia has less chance of being carried into the fertilized egg because of the number of mitoses that the affected cell must pass through before reaching the mature spermatozoon stage. Several investigators have reported that mitotic divisions of affected cells are checked (Strange-ways and Oakley, '23, Gatenby and Wigoder, '29, and others).

The close agreement in litter size observed between X-13-0 F_1 and its control is to be expected, since all the dominant lethals would have been eliminated in the first generation and recessives would not as yet have had a chance to meet. The X-13-0 F_2 in which effects of recessive lethals might be expected do not show any decrease in litter size. On the contrary, a slight increase is indicated.

The increase in percentage born dead noted in the immediate progeny of rayed males may be explained on the same basis as the decrease in litter size. Lethal effects as shown by Li ('27) in *Drosophila* may occur at various stages of development. The increase in percentage born dead noted in X-13-0 F_1 is difficult to explain on any genetic basis. No effect was observed in X-13-0 F_2 .

Birth weight shows a noticeable decrease. The difference between the control and the immediate offspring of rayed males was 2.70 times its standard error. The birth weights considered are of the young raised. Thus the effect on those born dead is not reflected. In X-13-0 F_1 no significant dif-

ference appears. A decrease is indicated in X-13-0 F_2 where recessives might be expected to produce effects.

The rather striking differences in thirty-day weights cannot with any confidence be ascribed to the effect of x-rays, since it may be an effect of an additional female in the pen.

A slight excess of males over females was obtained in the immediate progeny of rayed males. The ratio does not differ significantly from the control, but in view of the findings of Muller ('27) and Barth ('29), the possibility of a differential susceptibility of male- and female-producing sperm is pointed out. Parkes ('25) noted in the case of rats an increase of males in litters sired immediately following raying but later litters gave a striking predominance of females.

Of interest in connection with the present experiments are the recent studies on the effectiveness of various x-ray and radium dosages in the production of hereditary changes. Crowther ('25) pointed out the direct relation between biological effects and the degree of ionization produced by any given dosage. Stadler ('28) and Oliver ('30) have pointed out that the number of mutations produced is proportional to the dosage. Hanson, Heys, and Stanton ('31), measuring the degree of ionization produced by the dosages used, have concluded that the mutation rate is directly proportional to the ionization effects of the dosage. In the present experiments the trends noted with changes in dosage are, in general, in agreement with these results.

SUMMARY

1. Eighty-five males of a highly inbred strain of guinea-pigs (family 13) were treated with varied dosages of x-rays. A sterility period was induced and found to vary in duration and time of onset with dosage.

2. Seventy-one of the treated males were mated, producing a total of 173 offspring which gave 238 young in the second generations and 185 in the third. Larger numbers of control young were obtained during the same period from untreated animals of the same inbred stock.

3. The litters sired by the rayed males were definitely smaller in size than the control litters. The effect increased with the dosage.

4. The percentage dead in litters produced by the rayed males was considerably larger than in the control litters.

5. The young that reached the weaning age were slightly lighter at birth and at thirty days than the young of the control stock born in litters of the same size.

6. There was a suggestive but not certainly significant excess of males among the young.

7. The second and third generations showed no reduction in litter size compared with the controls. The second generation showed a rather high prenatal mortality and the third generation a rather light weight.

8. There can be little doubt that the reduction in the size of litter in the immediate progeny of the rayed males was an effect of the treatment. The most plausible interpretation seems to be the induction of dominant lethal mutations. The other effects are more doubtfully attributed to the treatment.

9. No visible mutations were observed among the progeny of the treated males, nor among the young of the second and third generations.

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